Male and Female Pubertal Protocols: Identifying Environmental Compounds That Alter The Thyroid axis

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Male and Female Pubertal Protocols

Applicability

Detects compounds that display:

anti-thyroid

Estrogenic

androgenic or anti-androgenic

altered puberty via changes in FSH, LH, PRL, GH or hypothalamic function.

anti-estrogenic [estrogen receptor (ER)]

steroid enzyme mediated activity

Questions

- Are the male and female pubertal assays useful as screens for thyrotoxicants with different mechanisms of action?
- Are results reliable?
  - What is Interlaboratory variability?

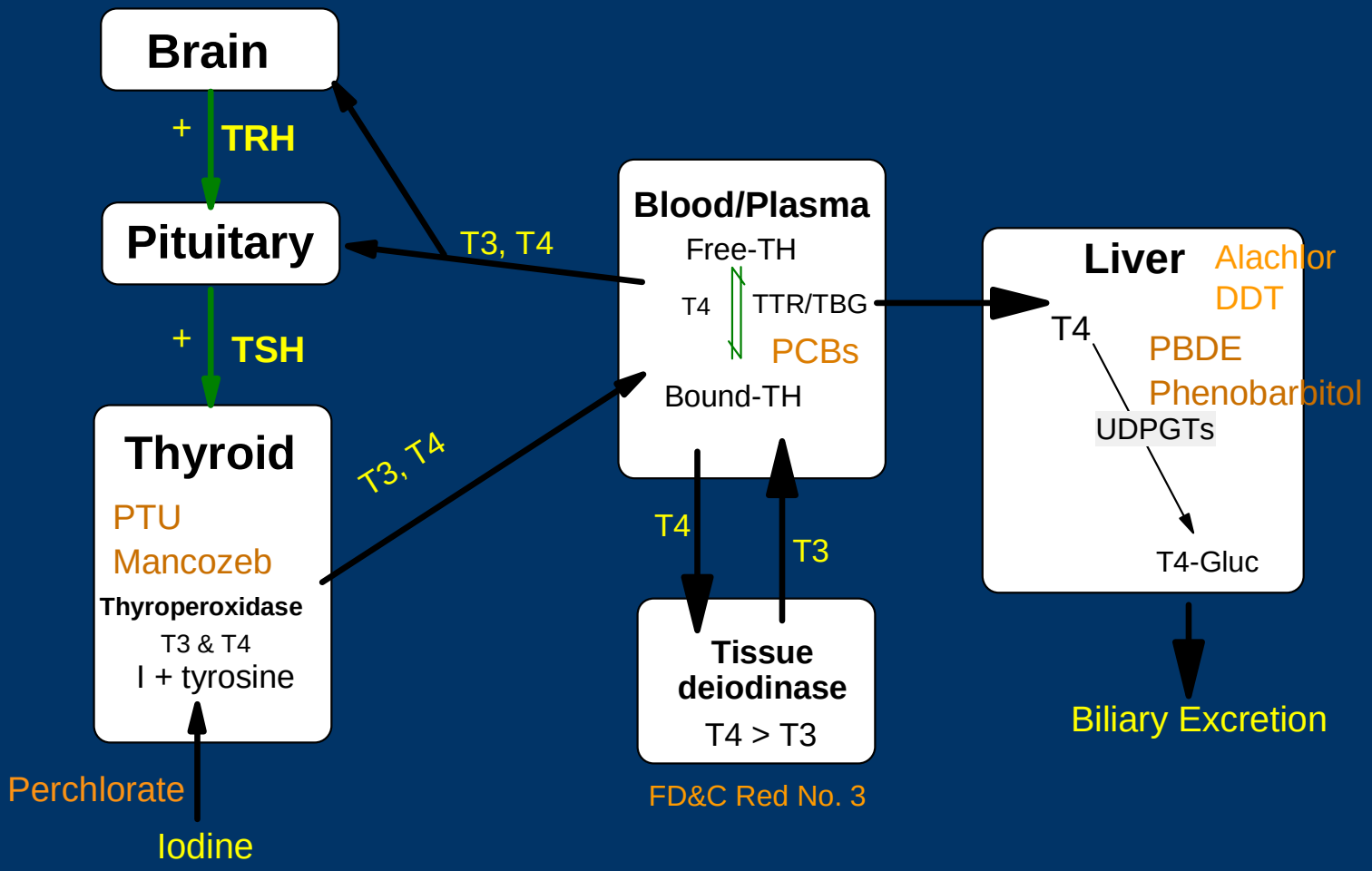
Overview

- Mechanisms of altered thyroid homeostasis.
- Examples of chemicals with specific mechanisms.
- Contract study results for PB and PTU
  - What was observed
  - Published studies vs. current data.
- New studies looking at other thyrotoxicants (EPA, ORD)

MECHANISMS OF ALTERED THYROID HOMEOSTASIS

- Clearance of thyroid hormones (ex. liver enzyme induction or altered TTR/TBG)
- Synthesis of thyroid hormones (ex. altered thyroperoxidase or altered uptake of iodine into the thyroid cell)
- Tissue deiodinase (peripheral conversion of T4>T3, FD & C Red No. 3, others?)
- Receptor Binding (??known)
- Brain/hypothalamic control of TRF (not known ??)

Mechanisms of Thyroid Disruption

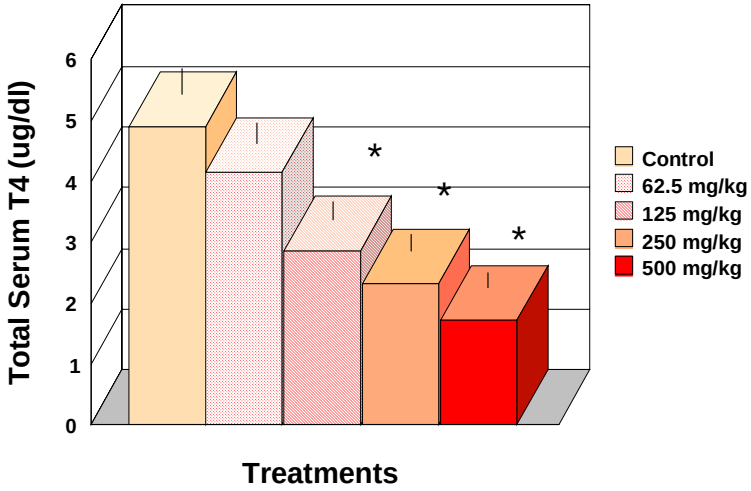


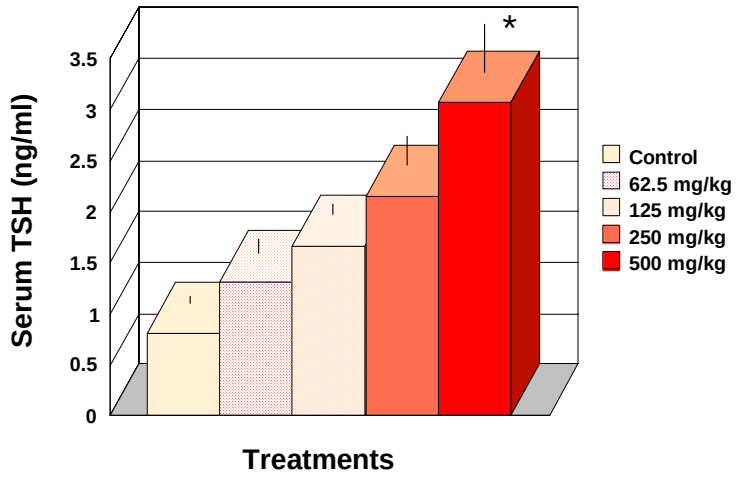
Altered Synthesis

EPA, RTP
Wistar female

Altered Thyroid Synthesis

- By altered KI symporter
  - Perchlorate

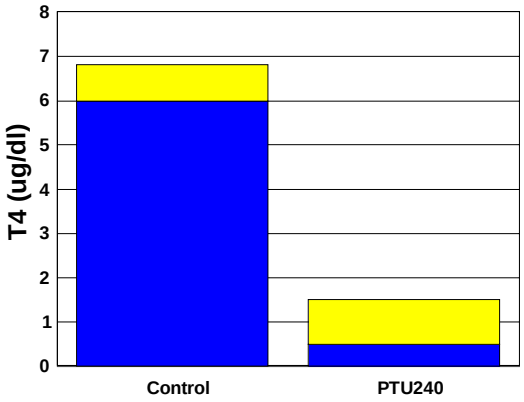


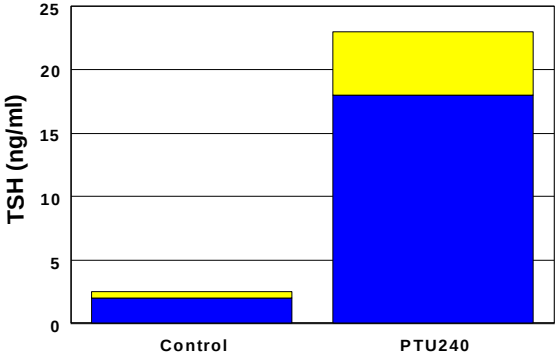


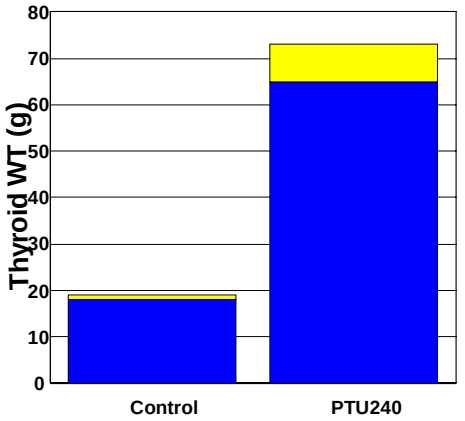
Marty et al.,
Tox. Sci., 2001) Male CD

Altered Synthesis of T4

- By inhibition of thyroperoxidase
  - PTU





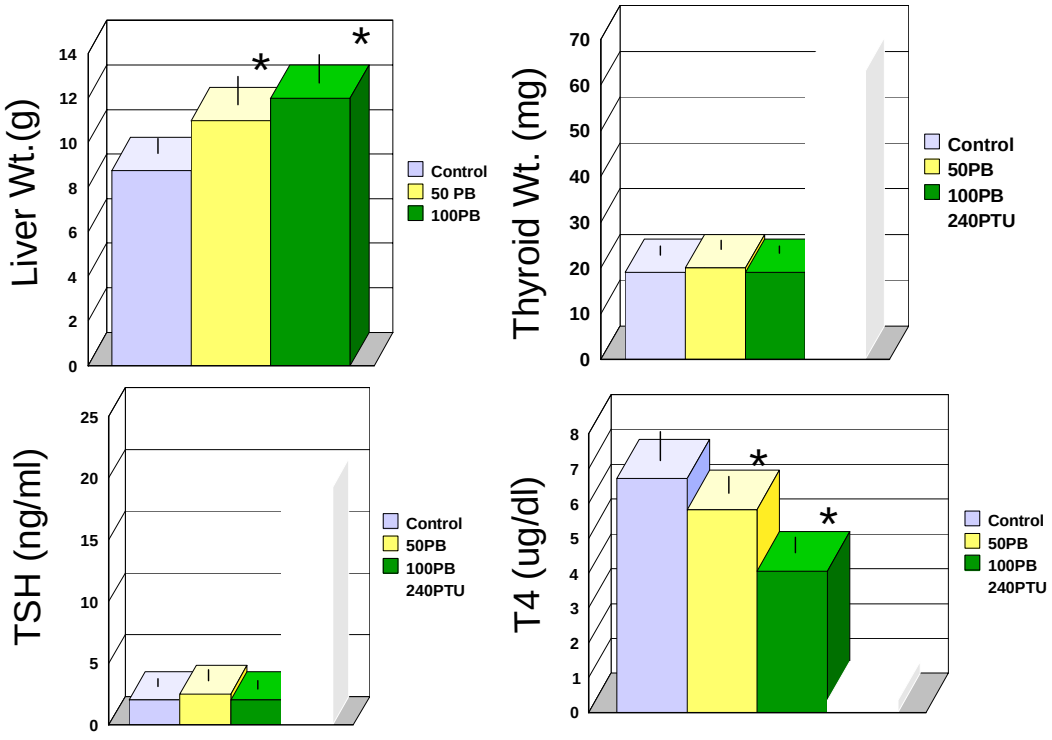


Altered Clearance of T4

Marty et al.,
Tox. Sci., 2001 Male CD

Altered Clearance of T4

- Phenobarbital in Pubertal Male



Pubertal Assays Detecting Thyroid Toxicants

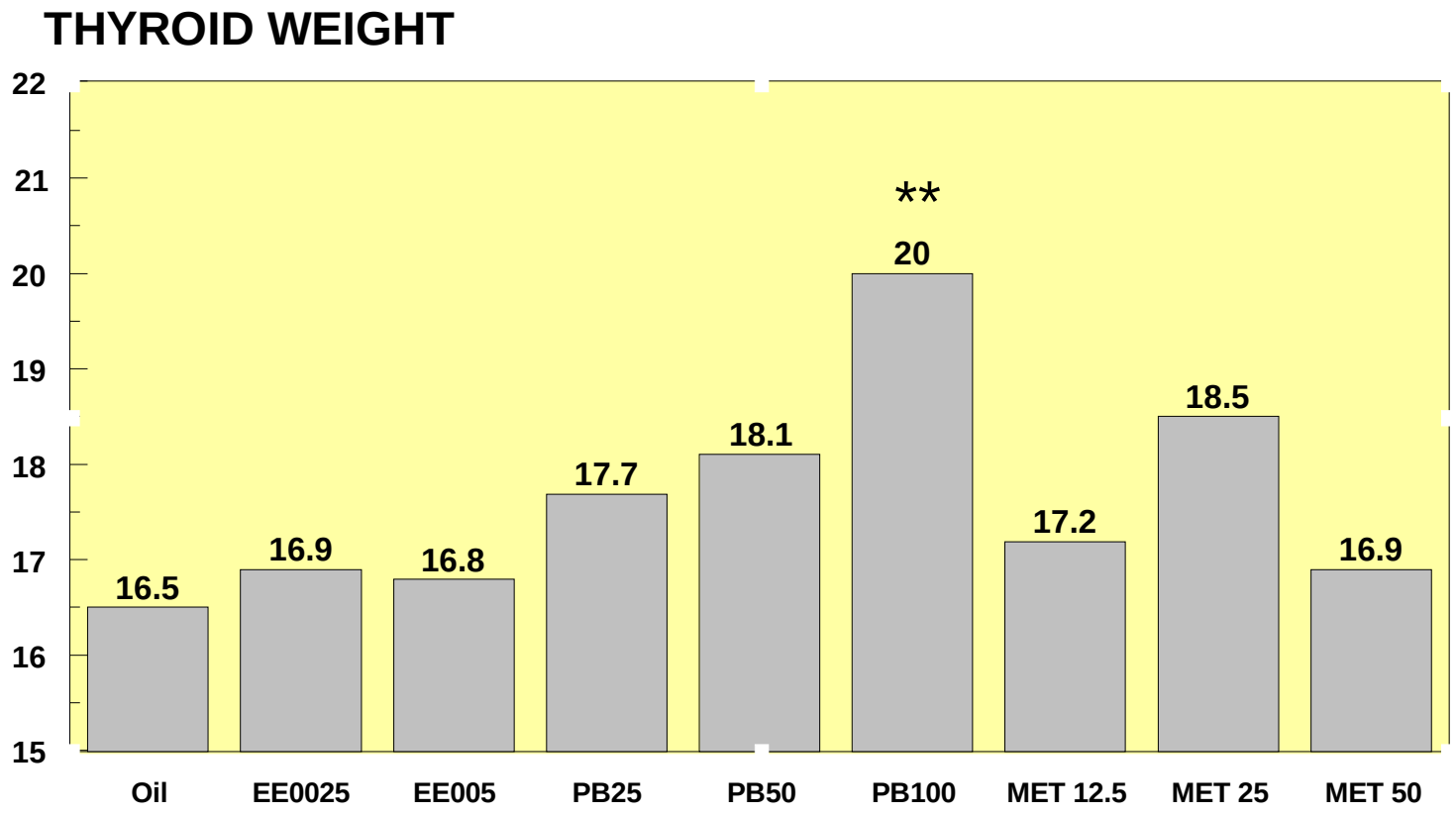
- Mechanism Identified by Assay(s)
  - Altered KI symporter
  - Altered thyroperoxidase activity
  - Altered clearance of T4
- Mechanisms not studied to date
  - Binding proteins (organochlorines, like OH-PCBs)
  - Deiodinase (FD&C Red No. 3)
  - Hypothalamic alterations

Results from Contract Studies

- Contract I, 2000 (PTU)
  - Thyroid Hormones
- Contract II, 2003 (PB)
  - Thyroid Weights
- Contract III, 2003 (PTU &PB)
  - Thyroid Hormones, Weights and Histology

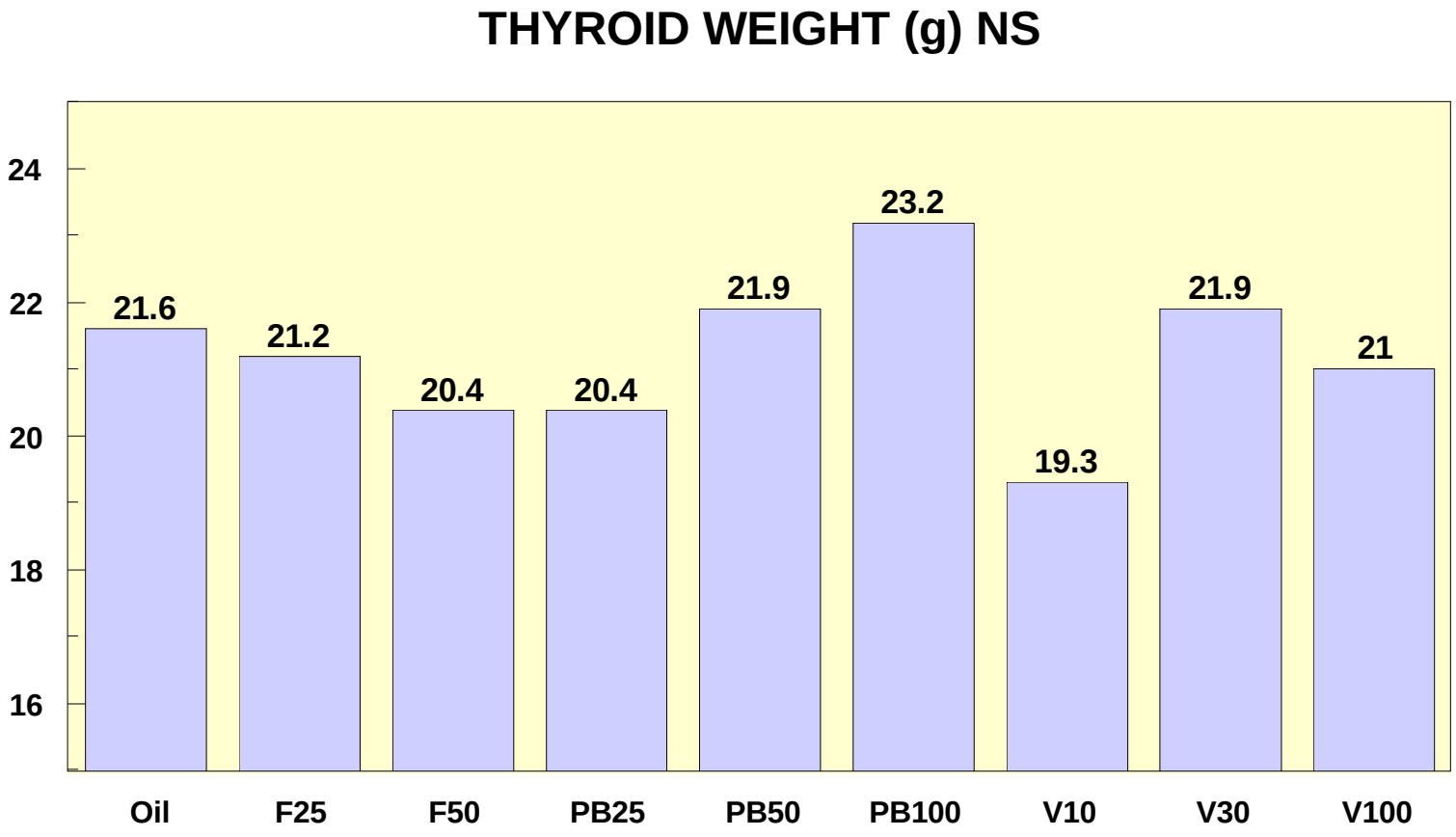
Contract Study II
2003

Effect of chemicals on Thyroid Weights in Female Pubertal



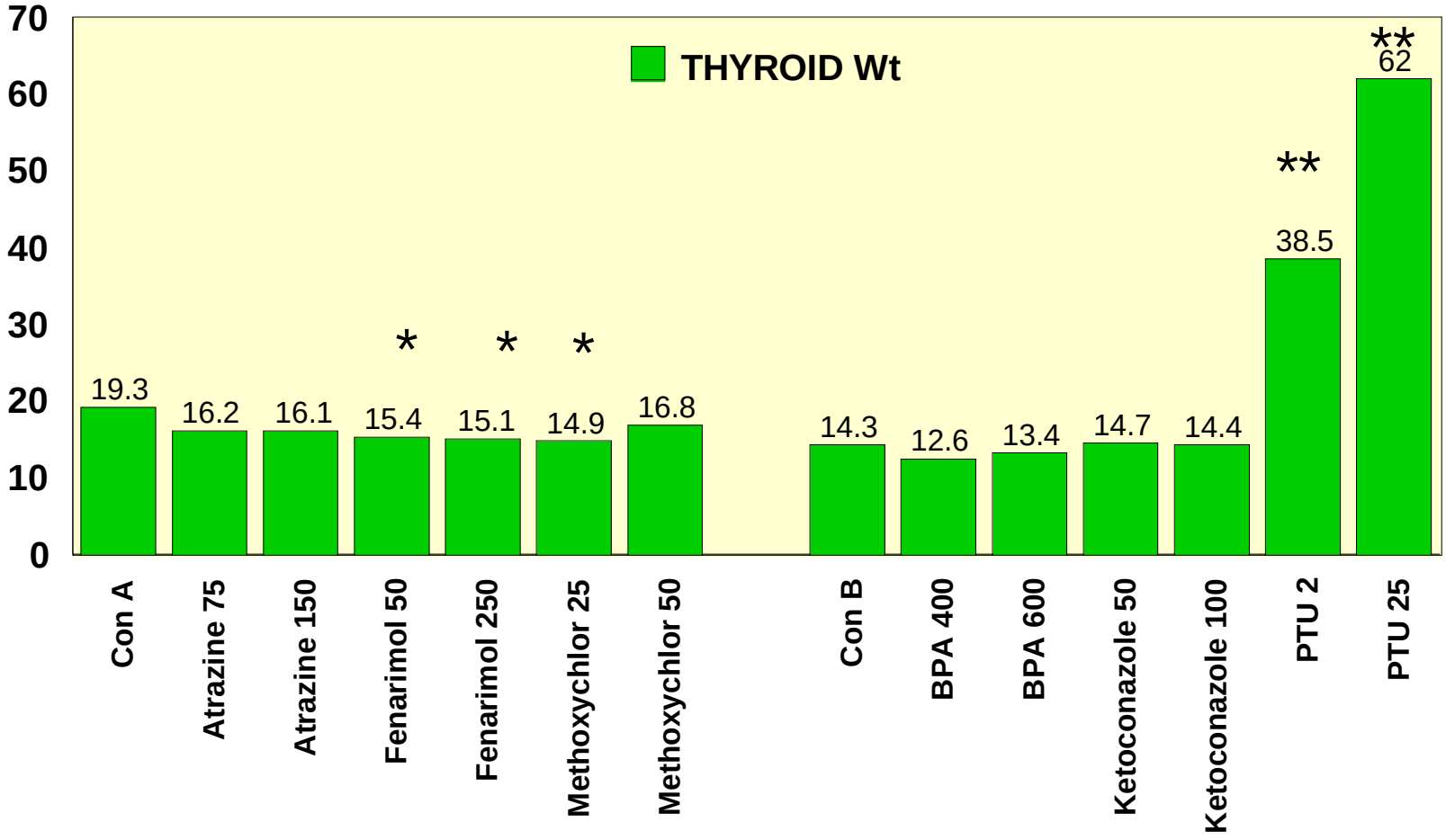
Contract Study II
2003

The effect of chemicals on thyroid weight in the Male Pubertal



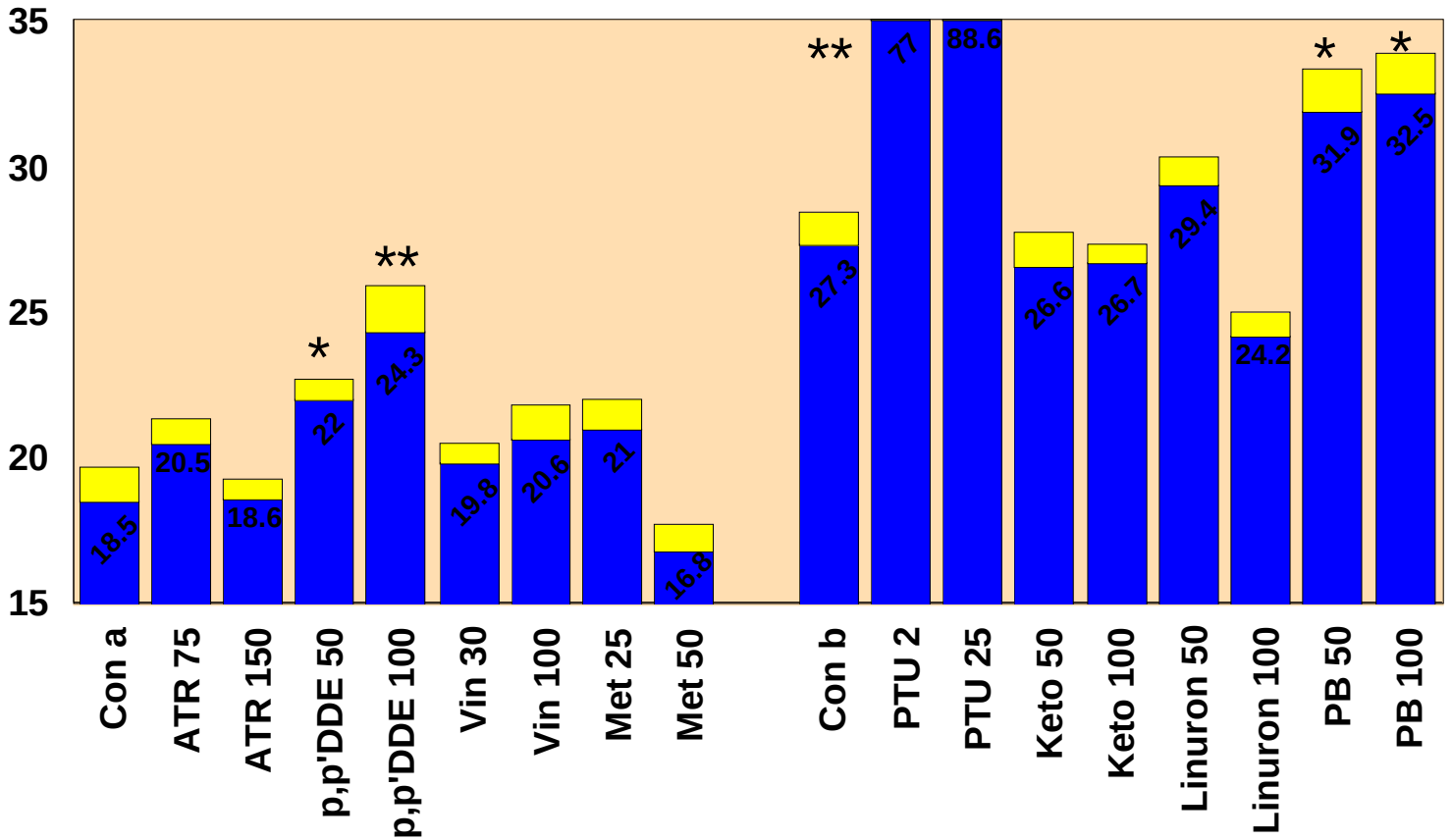
Contract Study III
2003

The effect of chemicals on thyroid weight in the Female Pubertal



Contract Study III
2003

The Effect of Chemicals on Thyroid Weight in the Male Pubertal.

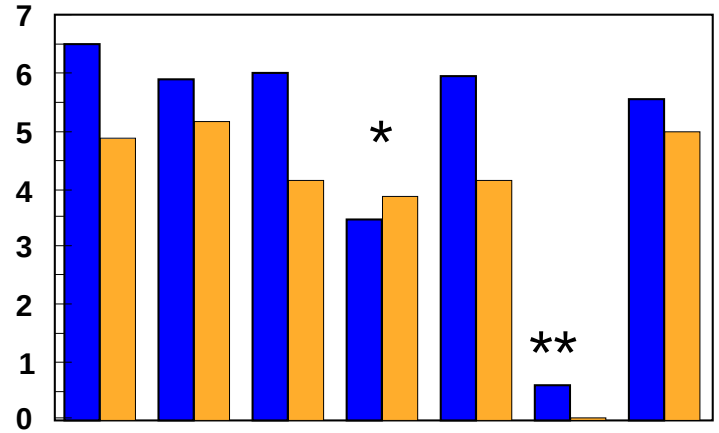


Contract Study I
2000

The effect of chemicals on thyroid hormones in the Male Pubertal

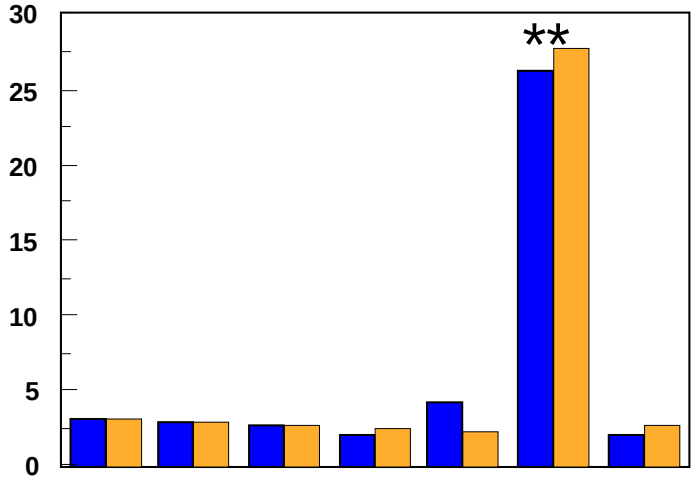
T4 ug/dl

LE>SD BUT NO INTERACTION WITH TI



	Oil	KETC	MT	DBP	FL	PTU	PIM
LE	6.54	5.88	6	3.45	5.97	0.6	5.58
SD	4.87	5.16	4.15	3.86	4.14	0	4.98

TSH ng/ml

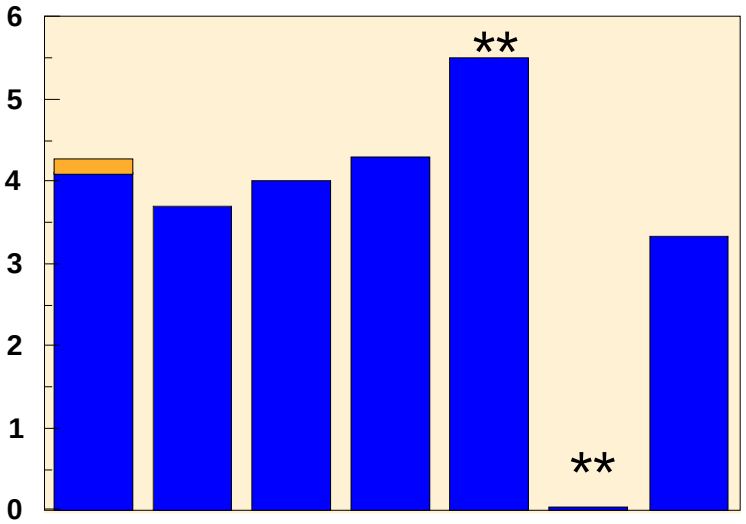


	Oil	KETC	MT	DBP	FL	PTU	PIM
LE	3.13	2.86	2.72	2.11	4.16	26.2	2.1
SD	3.06	2.98	2.67	2.43	2.36	27.7	2.68

Contract Study I
2000

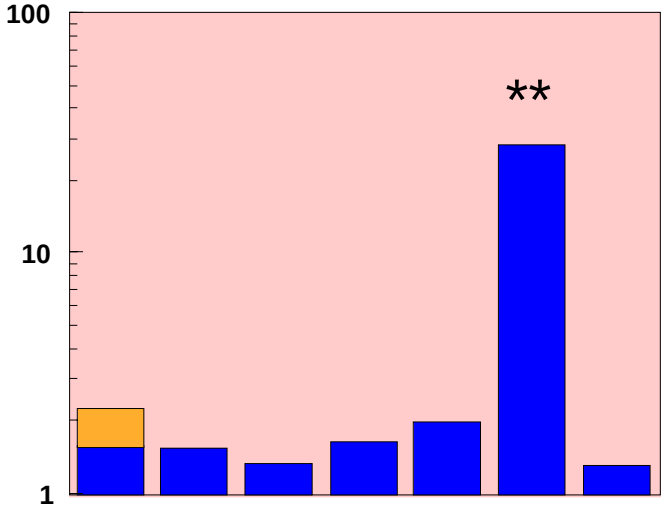
The effect of chemicals on thyroid hormones in the Female Pubertal

T4 ug/dl



Oil	KETO	MET	EE	TAM	PTU	PIM
4.12	3.71	4.01	4.3	5.51	0.03	3.34

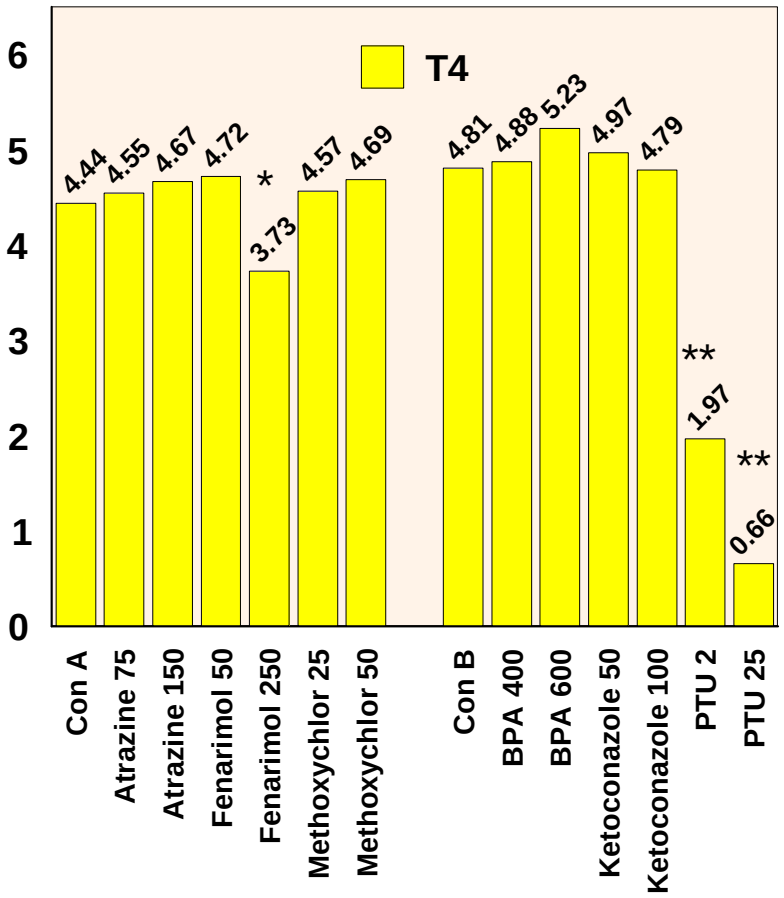
TSH ng/ml

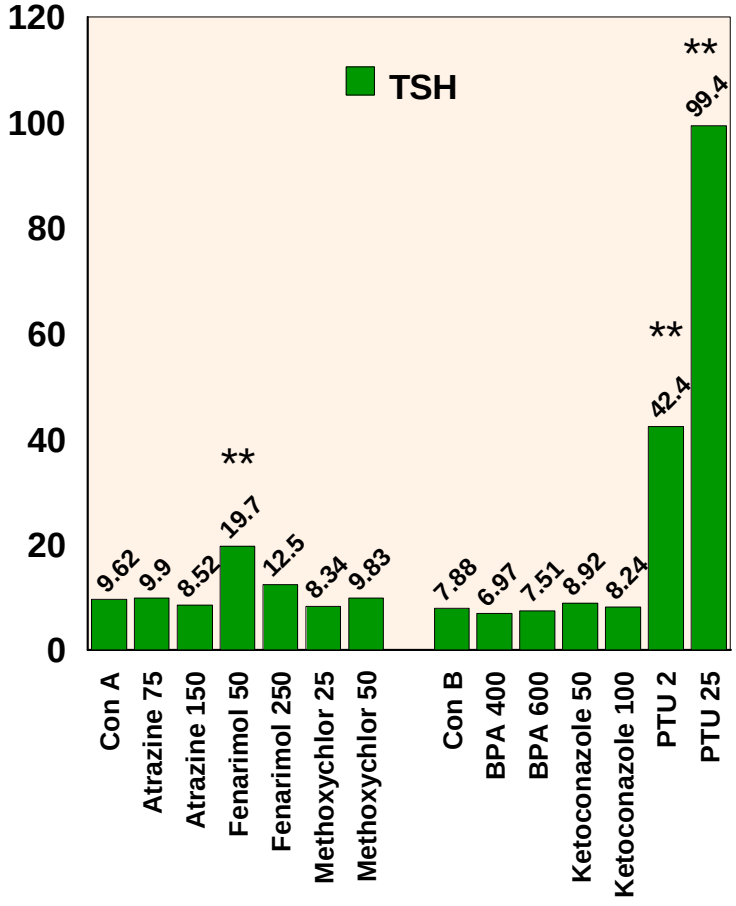


Oil	KETO	MET	EE	TAM	PTU	PIM
1.58	1.57	1.33	1.66	2	28.2	1.32

Contract Study III
2003

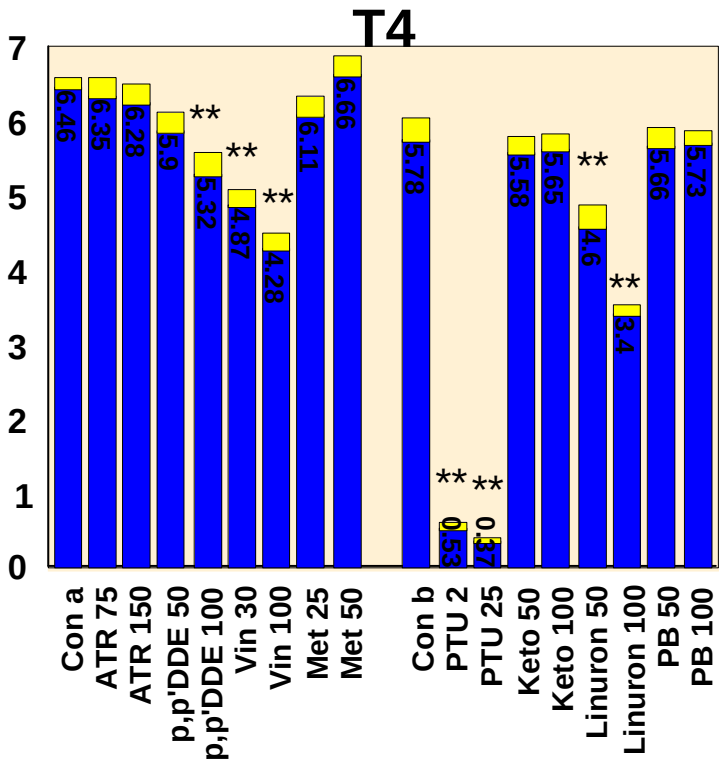
The effect of chemicals on thyroid hormones in the Female Pubertal.

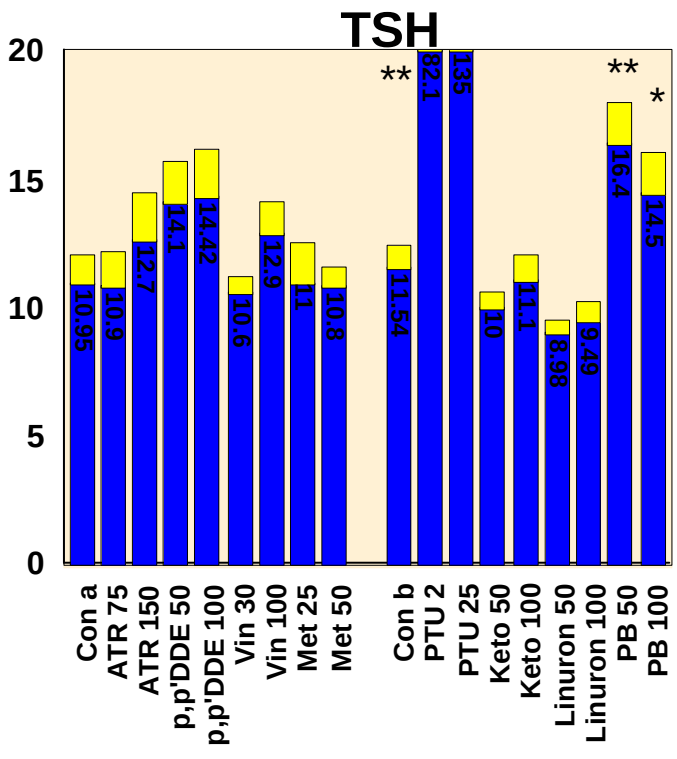




Contract Study III
2003

Effects of toxicants on Thyroid Hormones in the Male Pubertal





Summary of Contract Studies

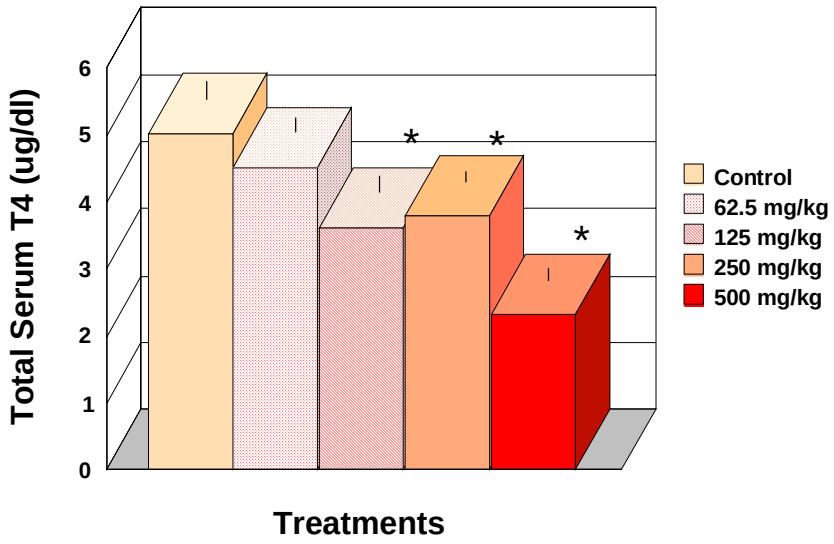
- All studies found alterations in thyroid parameters that were consistent with the chemical action
  - Thyroid Weights
    - Both Contract II and III found appropriate differences in weights, Contract I did not take weights.
  - Thyroid Hormones
    - PTU
      - Contract I and III identified PTU thyroid hormone effects (T4 and TSH)
      - Contract II did not test PTU
    - PB
      - Contract III identified changes in TSH, but no T4 decrease
      - Contract II did not measure hormones

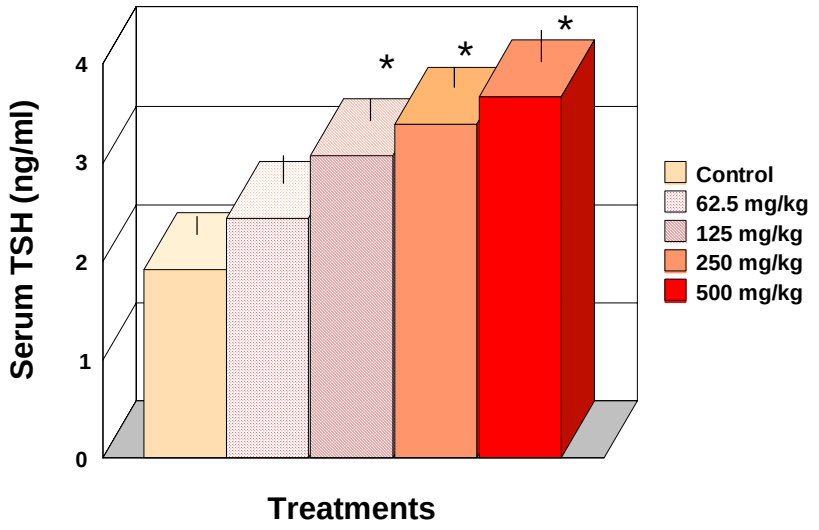
Identifying other chemicals (ORD studies)

- Perchlorate- KI symporter
- DE-71- Liver metabolism of T4

EPA Special Study
Wistar Rat

The Effect of Perchlorate on Thyroid Endpoints in the Male Pubertal

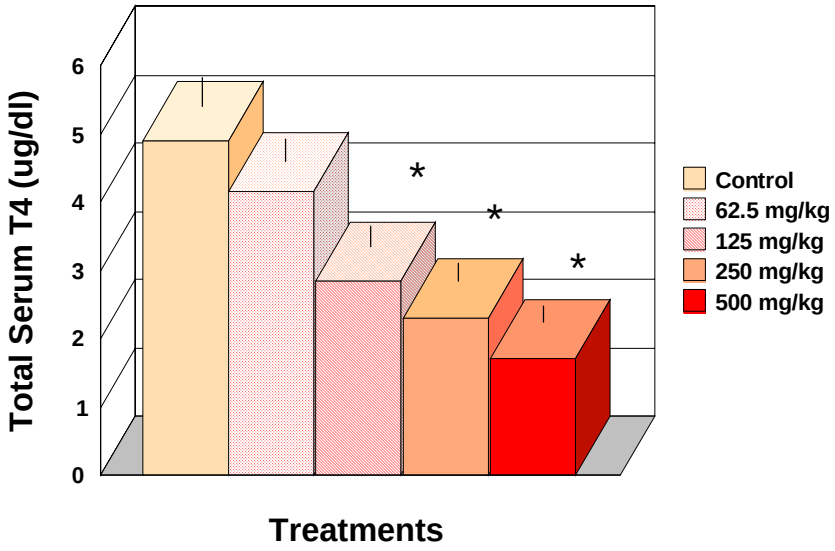


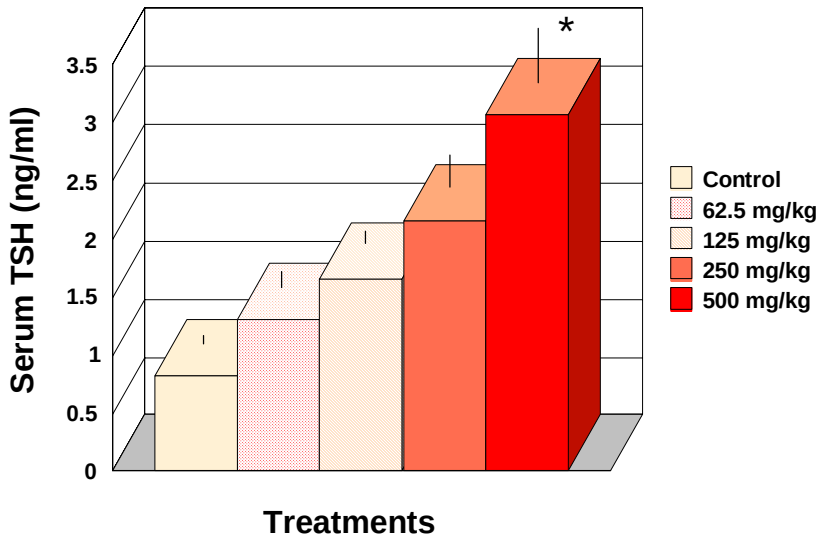


EPA Special Study
Wistar Rat

The Effect of Perchlorate on Thyroid Endpoints in the Female Pubertal Protocol

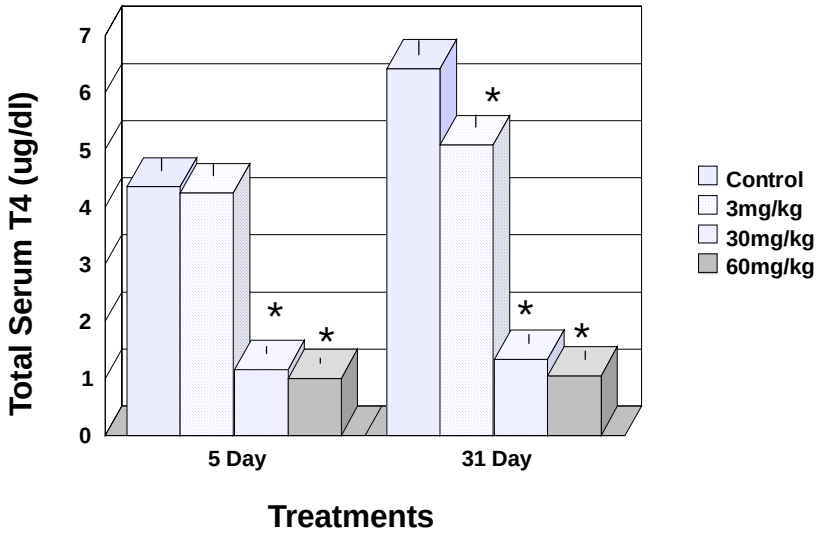
Females

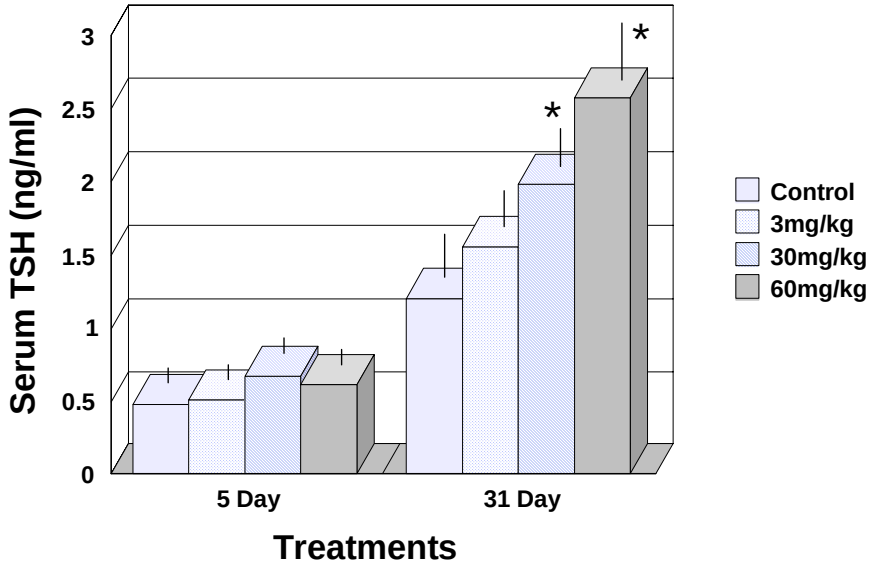




EPA Special Study
Wistar Rat

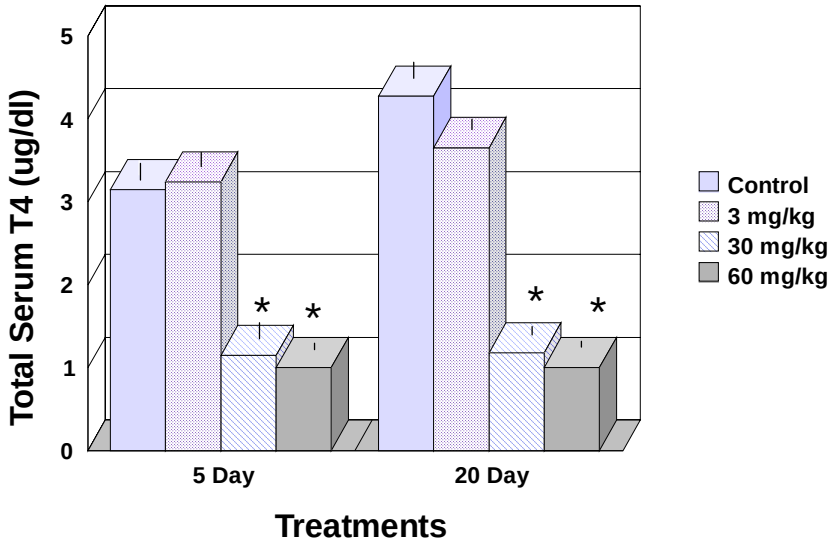
The Effect of DE-71 on Thyroid Endpoints in the Male Pubertal Protocol

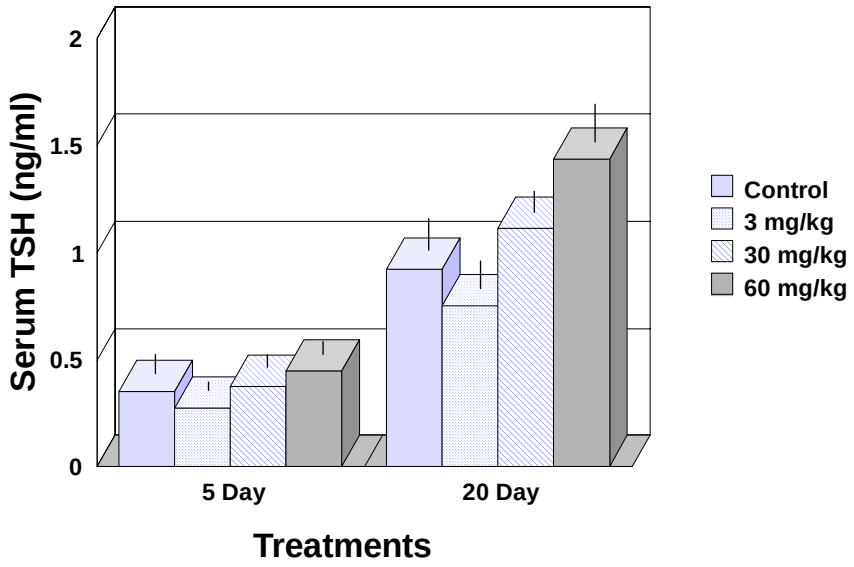




EPA Special Study
Wistar Rat

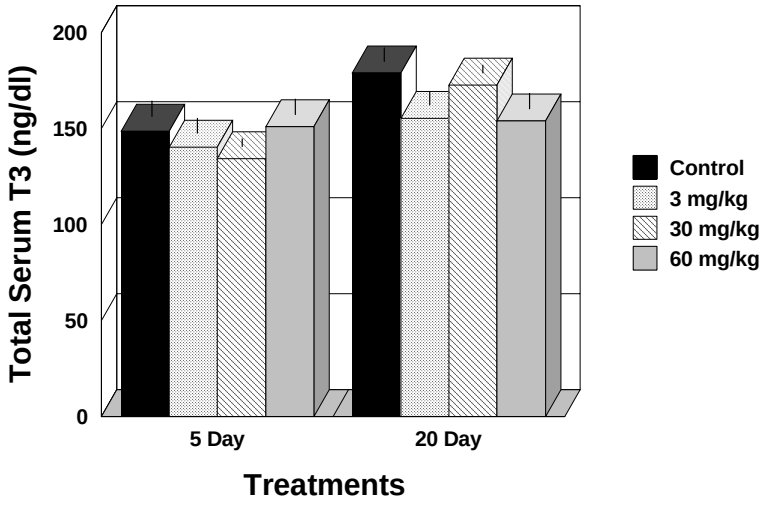
The Effect of DE-71 on thyroid endpoints in the Female Pubertal Protocol

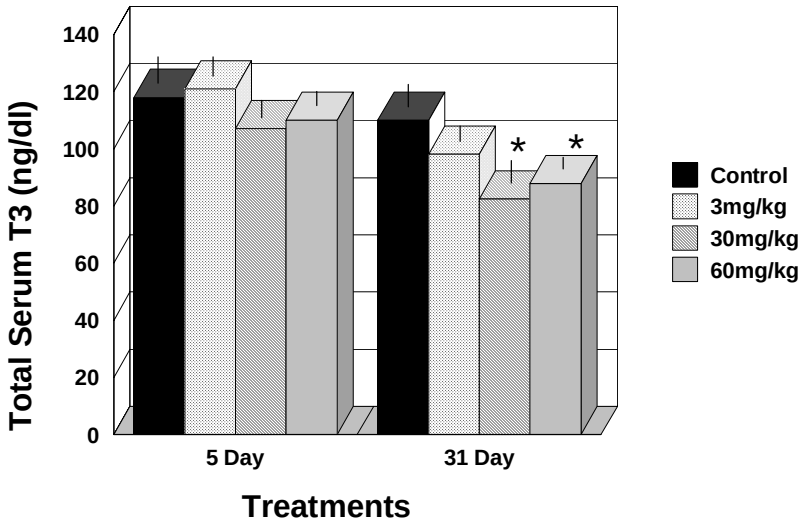




EPA Study
Wistar Rat

T3 in DE-71 Study
Comparison of Male and Female





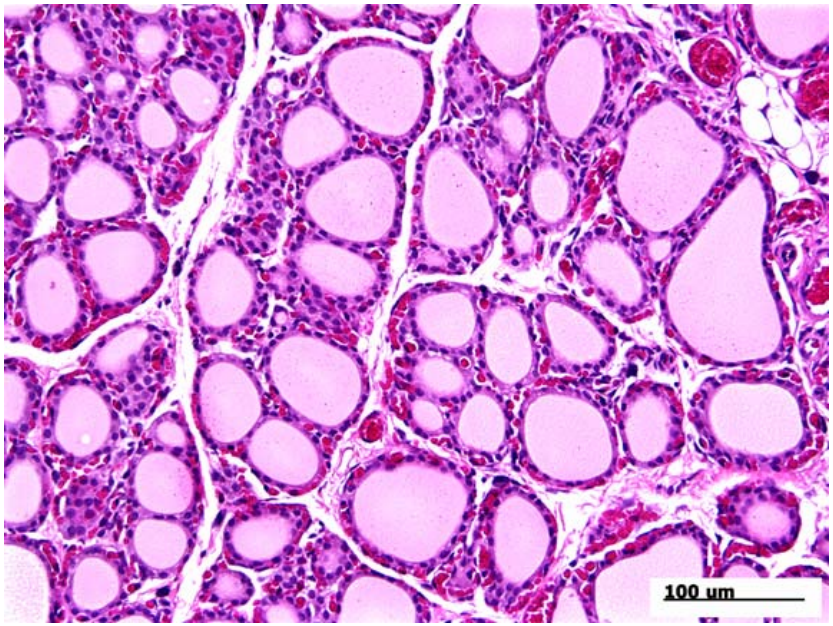
Thyroid Histopathology (hypo)

- Decreased Colloid Area
- Increased Follicular proliferation and cell heights.

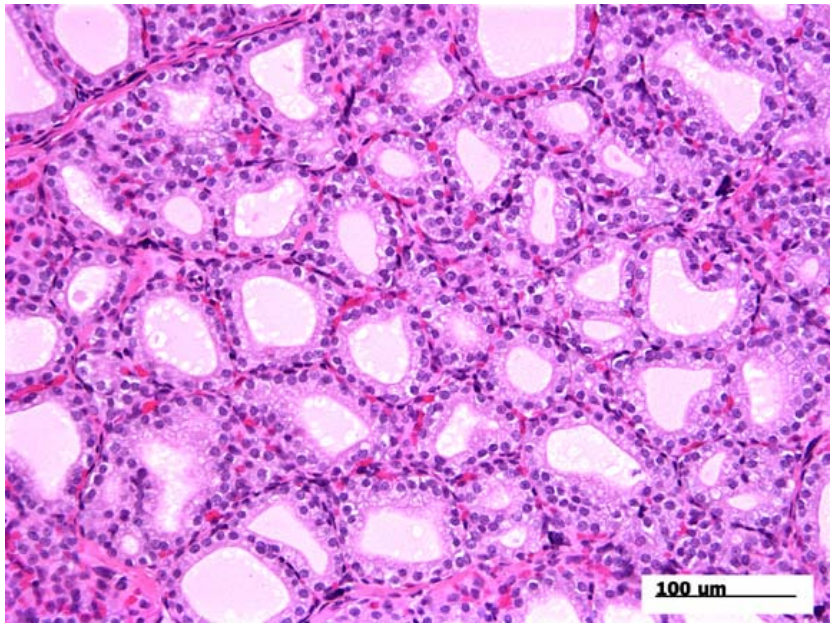
EPA DE-71 Study

Hypothyroidism

Control Male Thyroid



60 mg/kg DE-71 Male Thyroid

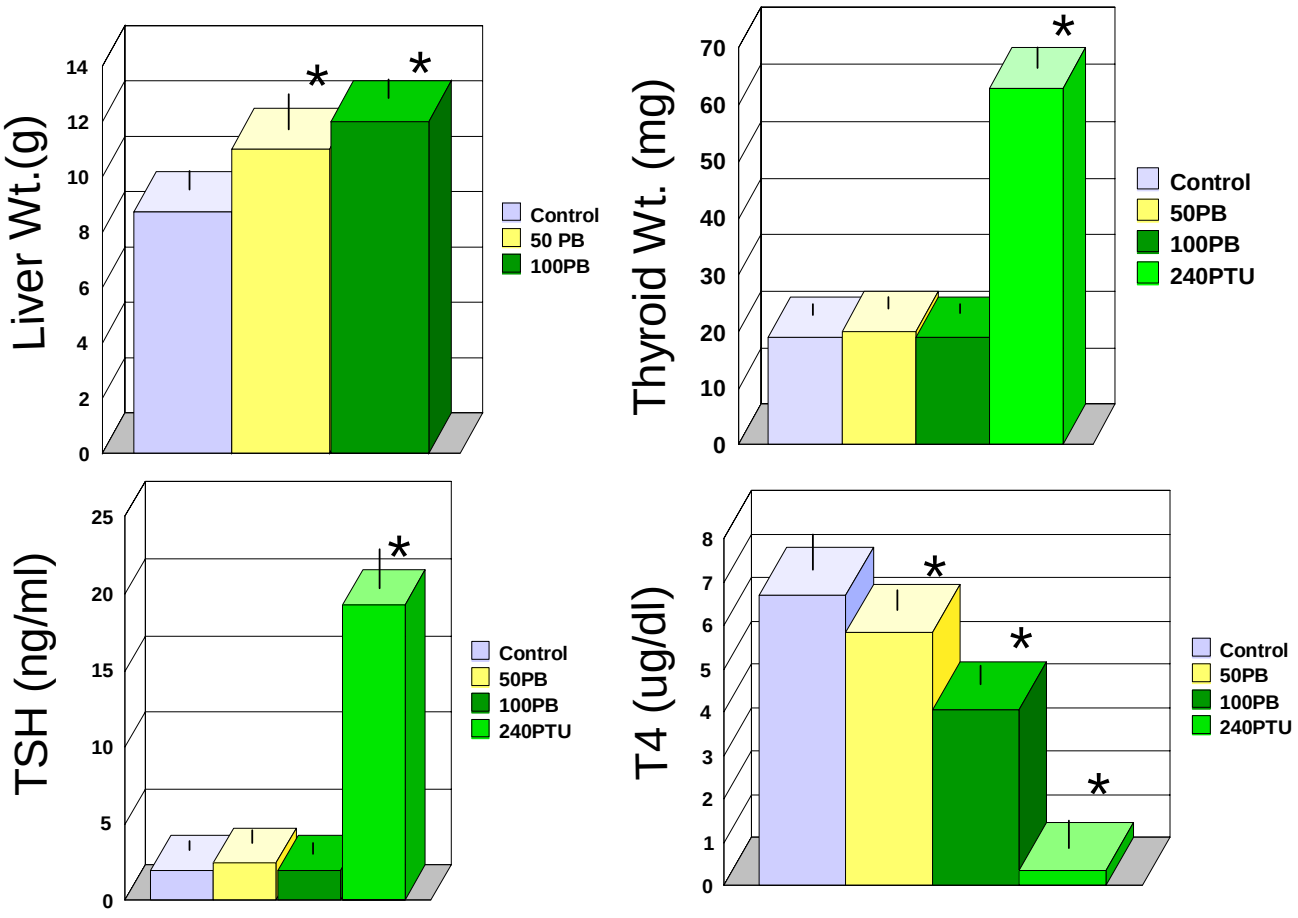


Also observed in female 60 mg/kg DE-71.

Conclusions

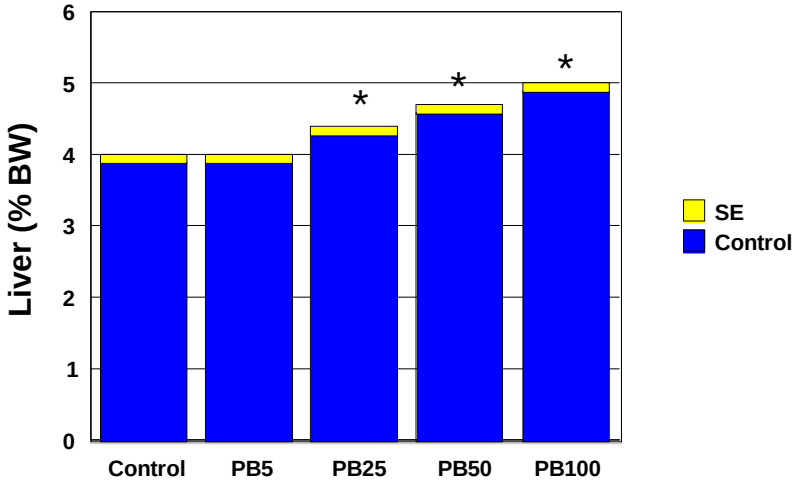
- Male and Female Pubertals.
  - No failures so far
  - 3 mechanisms examined
  - Do we need to do other mechanisms, before moving on? 5’ Deiodinase inhibitor, TTR, hypothalamus??
  - Some disappointments (maybe)
    - One contract lab missed PB effect on T4
      - Still thyroid effects observed! (TSH, liver, thyroid wt.)

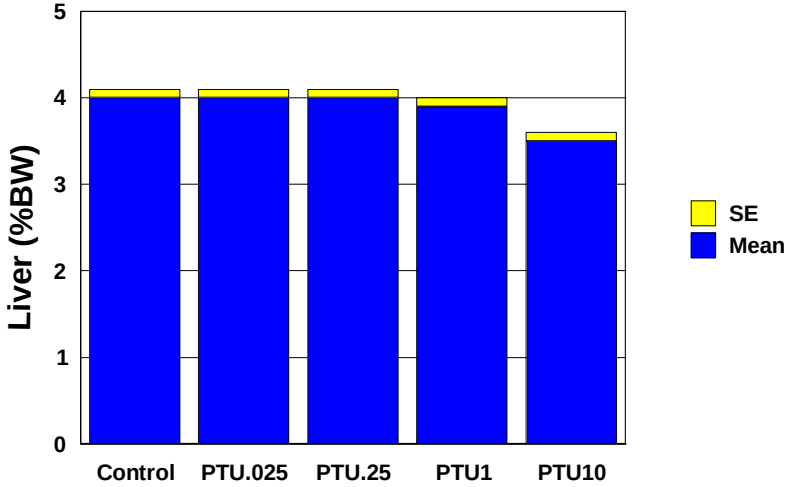
Marty et al., Tox. Sci., 2001)



O'Connor, 1999
15 Day SD Male

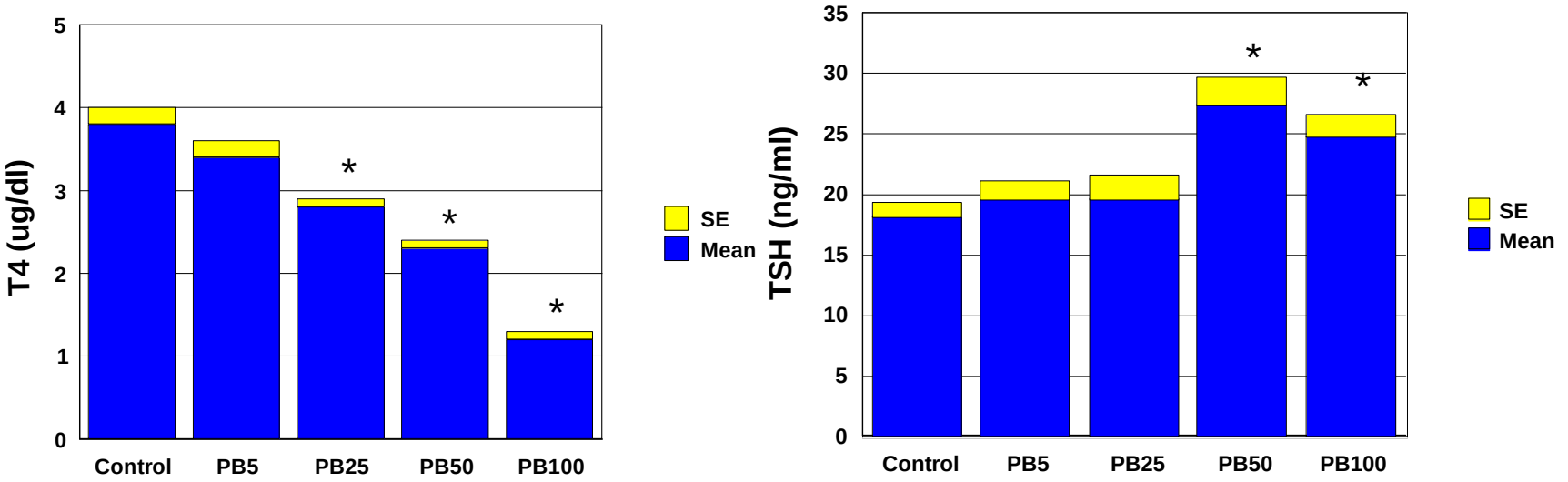
The Effect of PB and PTU on Liver in the Intact 15 Day Male Assay



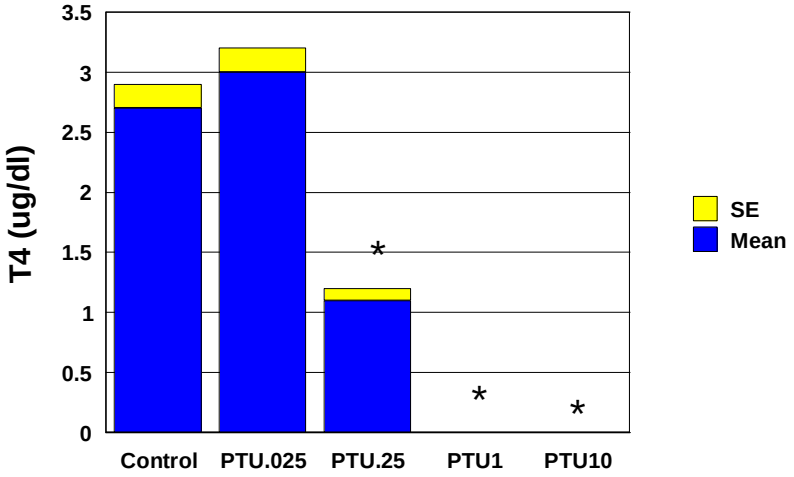


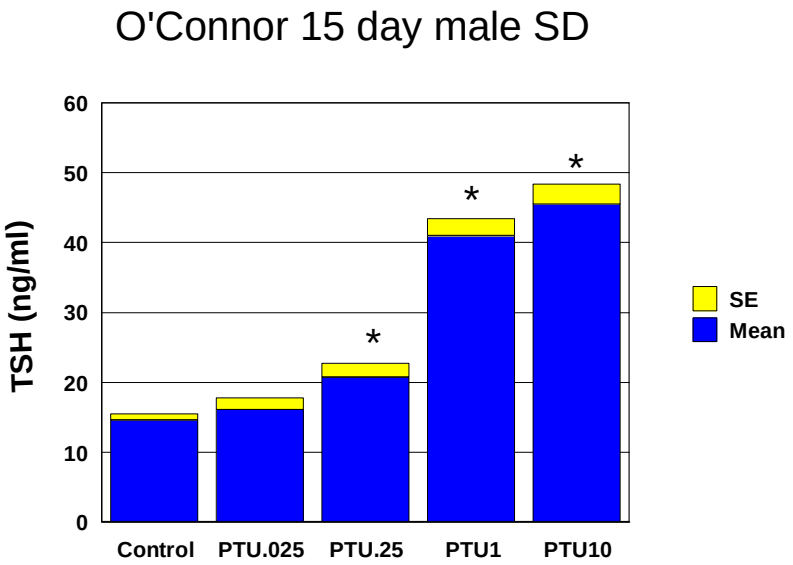
O’Connor, 1999

The Effect of PB on Thyroid Hormones in the 15 Day Intact Male SD



O’Connor


3.5
3
2.5
2
1.5
1
0.5
0
T4 (ug/dl)
*
*
*
Control
PTU.025
PTU.25
PTU1
PTU10
SE
Mean

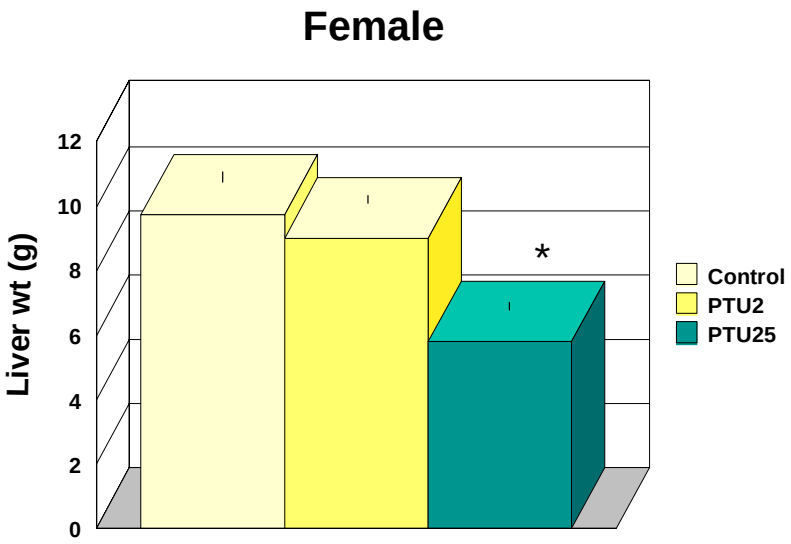

O'Connor 15 day male SD
60
50
40
30
20
10
0
TSH (ng/ml)
*
*
*
Control
PTU.025
PTU.25
PTU1
PTU10
SE
Mean

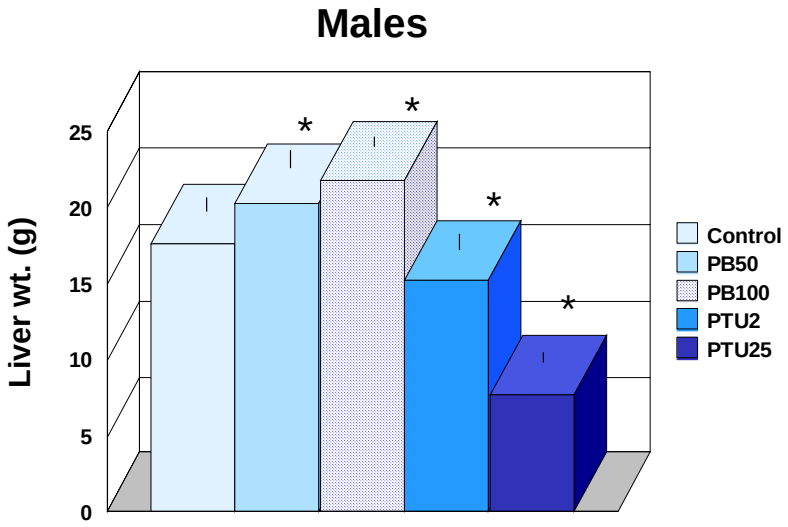
Studies

- Contract studies
  - Therimmune 2000
    - PTU (240) , male and female in SD and LE
  - Therimmune 2003
    - PB (25, 50, 100 mg/kg) male and female SD
  - RTI
    - PTU (2 and 25 mg/kg) male and female SD
    - PB (50 and 100 mg/kg) male and female SD
- EPA studies
  - PBDE and perchlorate.
- Industry studies
  - Marty et al., PB 50 and 100 mg/kg and PTU 240 mg/kg in male SD
  - O'Connor et al., PB 5, 25, and 50 mg/kg or PTU .025, .25, 1 and 10 mg/kg in SD males (15 day intact)
  - Yamada, PB at 125 mg/kg and PTU at 2.5 mg/kg (10 day HERSH)

Contract Study III
2003

The effect of chemicals on liver weight in the Male and Female Pubertal

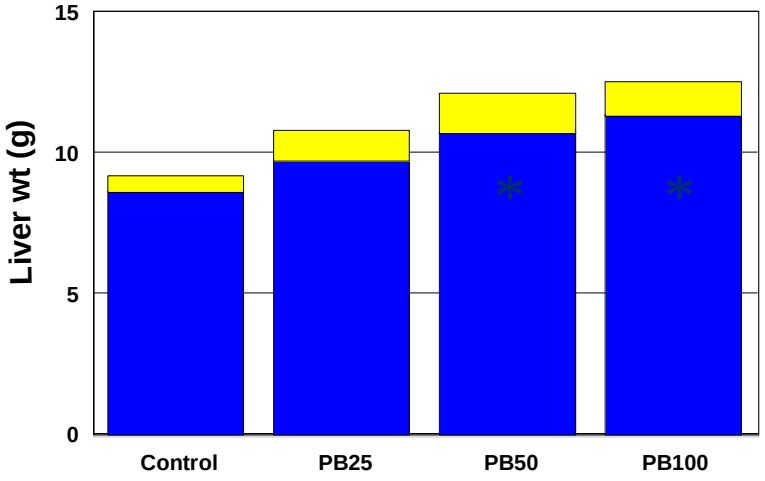




Contract Study II
2003

Effect of chemicals on liver weight in Male and Female Pubertals

Female



Male